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Human Albumin Solders for Clinical Application During Laser Tissue Welding

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Background and Objective: **Fifty percent human albumin solder significantly improves weld strength when compared to lower concentrations [Wright et al., ASLMS meeting, April, 1995]. We developed a method for preparing 50% human albumin that may be considered compatible for clinical applications.**
Study Design/Materials and Methods: **Fifty percent human albumin solder was prepared from 25% commercially available human albumin using a lyophilization technique. Assessment of sterility, viscosity, pH, and peak absorption wavelength were performed.**
Results: **This report describes the methology used to prepare a 50% human albumin solder that is compatible with clinical use. Maintenance of the structural integrity of the albumin was confirmed by polyacrylamide gel electrophoresis.**
Conclusion: **This solder preparation can be used alone or with the addition of exogenous chromophores. The final product is sterile, incorporates viral free protocols, maintains high viscosity, and can be applied easily during open or laparoscopic procedures.** © 1996 Wiley-Liss, Inc.

Key words: chromophore, gamma radiation, lyophilization, polyacrylamide gel electrophoresis, sterilization, viscosity

INTRODUCTION

In 1985, our lab initiated the first studies to incorporate the technique of laser tissue welding for urethral reconstruction. The purpose of this research was to develop a method of tissue approxamation that could be used to reduce the 6–30% postoperative urethrocutaneous fistula rate that can occur following hypospadias repair [1]. Initial results suggested that the use of laser energy alone was insufficient to produce a weld for the repair of urethral tissue. These results led us to investigate novel methods to strengthen the weld site by applying different biological substances to act as a "solder" during laser welding. This research lead to the development of the first albumin solder for laser tissue welding [2]. When this solder was used during the repair of urethral defects a significant improvement in wound healing, reduced fistula rate, and decreased operative time were demonstrated [3]. More recently, numerous studies have confirmed the use of albumin as a suitable agent for enhancing the laser weld [4–9].

In 1993, our group published a review of the brief history of laser tissue solders and a method for preparation of a 40% human albumin solder that would be suitable for clinical use [10]. Recently, human albumin solder has been modified by adding exogenous chromophores to enhance the absorption of specific wavelengths of light [11,12].

As the clinical applications for laser tissue welding develop, it will be important to identify the solder concentration that provides the strongest weld, at the same time remaining free from bacterial and viral contamination. We have re-

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cently completed a study that indicates a significant improvement in weld strength when a 50% human albumin solder is employed compared to lower concentrations [13]. In this study, 1 cm longitudinal full thickness defects in the canine ureter were repaired using 25, 38, 45, and 50 percent solutions. Results were significant ($P < .0001$, ANOVA) with higher albumin concentrations yielding improvement and reliability in acute weld strength.

This report describes a method for preparing a 50% human albumin solder that incorporates viral free protocols, maintains high viscosity and sterility, and can be applied easily during open or laparoscopic procedures.

MATERIALS AND METHODS

Solder Preparation

All procedures practiced in the preparation of this solder were performed using sterile techniques. Manipulation of all containers and fluids was performed in a laminar flow hood. It was important to record the lot number of the stock albumin solution on all subsequent containers. If necessary, this number can be used to trace the final solder solutions back to the original blood donors.

For making a 50% concentration of albumin, 25 ml of 25% commercially prepared human albumin (Albuminar-25, Armour Pharmaceutical Company, Kankakee, IL) were transferred to a 50 ml conical tube (Becton Dickinson Labware, Lincoln Park, NJ.). The tube was frozen to minus 80°C for a minimum of 2 hours. During the freezing process, the tube was placed at a 30° angle to increase the surface area of the albumin exposed to air. This improves the rate of freeze drying later in the preparation. Once freezing was complete, the screw cap on the tube was removed and replaced with a sterile silicone sponge air filter cap (Sigma Chemical Co., St. Louis, MO). This filter cap maintains sterility of the albumin during the lyophilization process. The albumin was lyophilized using a Virtis 12-SL lyophilizer (Virtis Co., Gardiner, NY) for a minimum of 16 hours. The lyophilized albumin was reconstituted to a final volume of 12.5 ml (one-half the original volume) by adding 8.0 ml of sterile water. After performing numerous reconstitutions, it was determined that by adding 8.0 ml of water to a 25 ml lyophilized sample, a final volume of 12.5 ml was obtained. The average weight of the 25 ml of lyophilized albumin was 6.44 grams (±.05 grams). By reconstituting the albumin to one-half of the original volume, the concentration was doubled. This was confirmed by protein assay described below. pH of the final solution was determined using a Corning model 220 pH meter (Corning, Corning, NY).

One milliliter aliquots of the albumin solution were separated into 3 ml red top vacutainer tubes (Becton Dickinson). A vacuum was created in the tube by removing 1 ml of air with a tuberculin syringe and 25 gauge needle.

The tubes were individually placed into self-sealing sterilization bags along with a 1 ml syringe, 18 gauge needle (Becton Dickinson) and 20 gauge blunt tip needle (Baxter Scientific Products, McGaw Park, IL). The bags were transferred to a Cis Bio International Model IBL-437C gamma irradiator (ORIS Industries, France) and irradiated with 36.4 kGy (3640 rads). The dosing was derived from the Guideline for Gamma Radiation Sterilization (Association for the Advancement of Medical Instrumentation, Arlington, VA). Assuming a bioburden (number of organisms) of 1,008,900 and a <1 in 1,000,000 chance of having a nonsterile sample, the radiation dose was calculated to be equal to 36.4 kGy. Following sterilization, the samples were stored at room temperature until they were used. The final albumin solutions have been tested for sterility by plating the albumin onto culture dishes containing Luria-Beertani broth and incubated for 72 hours at 37°C in a Lab-Line model 3526 incubator (Lab-Line Instrument Co., Melrose Park, Ill.)

Initial attempts to use cold filter sterilization incorporating various pore size filtration steps leading to a final 0.2 μm filtration, could not be performed for solder concentrations above 35%. Concentrations above this level resulted in blockage of the filter system and increased the volume of albumin wasted or trapped in the filtration process.

In the operating room, the sterile bag can be opened onto the surgical field. The 18 gauge needle can be used to draw the solder into the syringe. The needle should be immediately discarded and replaced with the blunt tip needle. The use of a blunt tip needle decreases potential injury to the surgical staff and to the host tissues at the site of solder application.

Chromophore Enhanced Solder Preparation

Indocyanine Green (MW 774.96) (used with 808 nm diode laser): A 0.54 mM solution of ICG albumin solder was prepared by adding

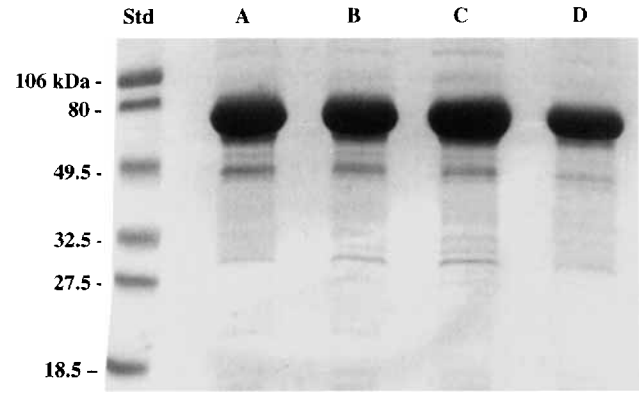

Fig. 1. Polyacrylamide gel electrophoresis of human albumin solders run under denaturing conditions to analyze primary structural changes during solder preparation. (STD) Protein standard, (A) 25% human albumin control, (B) 25% human albumin after freezing to −80°C, (C) 50% human albumin reconstituted from lyophilized powder, (D) 50% human albumin reconstituted from lyophilized power and gamma irradiated with 5,000 rads. Banding patterns confirm primary structure of protein remain intact.

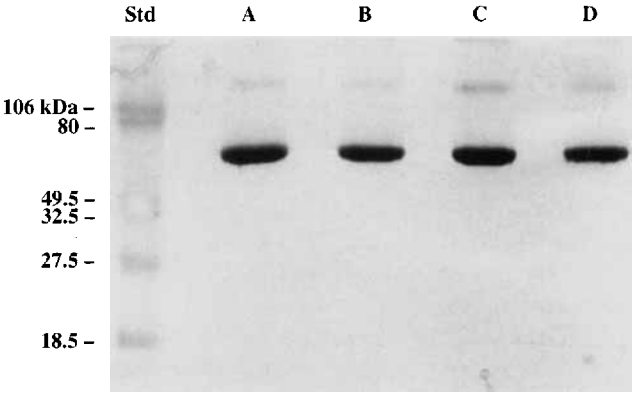

Fig. 2. Polyacrylamide gel electrophoresis of native (not denatured) human albumin solders analyzing the secondary and tertiary protein structure. (STD) Protein standard, (A) 25% human albumin control, (B) 25% human albumin after freezing to −80°C, (C) 50% human albumin reconstituted from lyophilized powder, (D) 50% human albumin reconstituted from lyophilized power and gamma irradiated with 5,000 rads. Banding pattern confirms maintenance of secondary and tertiary structural integrity.

0.42 mg of indocyanine green (Cardiogreen, Sigma) to each milliliter of the final 50% albumin solution. The ICG was solubilized in the distilled water that was used to reconstitute the lyophilized albumin. It was important to completely dissolve the ICG in water prior to adding it to the albumin. This prevents clustering of the ICG.

Fluorescein (MW 332.3) (used with 532 nm diode laser): A 0.54 mM solution of fluorescein solder was prepared by adding 1.79 ml of 100 mg/ml fluorescein (Alcon Laboratories, Fort Worth, TX) to each milliliter of the final solder solution. The volume of fluorescein used was subtracted from the total volume of distilled water needed to reconstitute the lyophilized albumin.

Methylene Blue (MW 373.90) (used with 670 nm diode laser): A 0.54 mM methylene blue solder was prepared by adding 20.2 ml of 10 mg/ml stock methylene blue (Star Pharmaceuticals, Pompano Beach, FL) per milliliter of final solder volume. This volume of methylene blue was subtracted from the total volume of distilled water that was used to reconstitute the lyophilized albumin.

Spectrophotometric analysis of all solders was accomplished using a Hewlett Packard Model 8452-A diode array spectrophotometer (Hewlett Packard Co., Palo Alto, CA). This analysis was performed to determine the peak absorption wavelength for each chromophore enhanced solder and to verify that no shift in peak absorbance occurred when the chromophore was added to the 50% albumin solder.

Polyacrylamide Gel Electrophoresis

Aliquots of solder were evaluated by polyacrylamide gel electrophoresis (PAGE) under denaturing (i.e., in the presence of reducing agents) and native conditions [14]. Samples of solder evaluated included a 25% commercial human albumin as a control, 25% albumin frozen to −80°C and thawed to room temperature, 25% albumin lyophilized, and reconstituted to 50%, and 50% albumin gamma irradiated with 5,000 rads. The sample loading buffer for the denaturing gels contained sodium dodecyl sulfate (SDS) and beta-mercaptoethanol, whereas that for the native gels lacked these components. Equal amounts of each protein solution were electrophoresed on 12% gels under denaturing and native (nondenaturing) conditions. Gels were stained with colloidal Coomassie brilliant blue and air-dried between cellophane sheets [15]. The resulting protein banding patterns were visually evaluated to determine if denaturation or fragmentation of the albumin had occurred during the preparation of the 50% solder.

Measurement of Viscosity

Viscosity of 25, 40, and 50% human albumin solders was determined. The measurements were obtained using the Zahn Cup-Type Viscometer

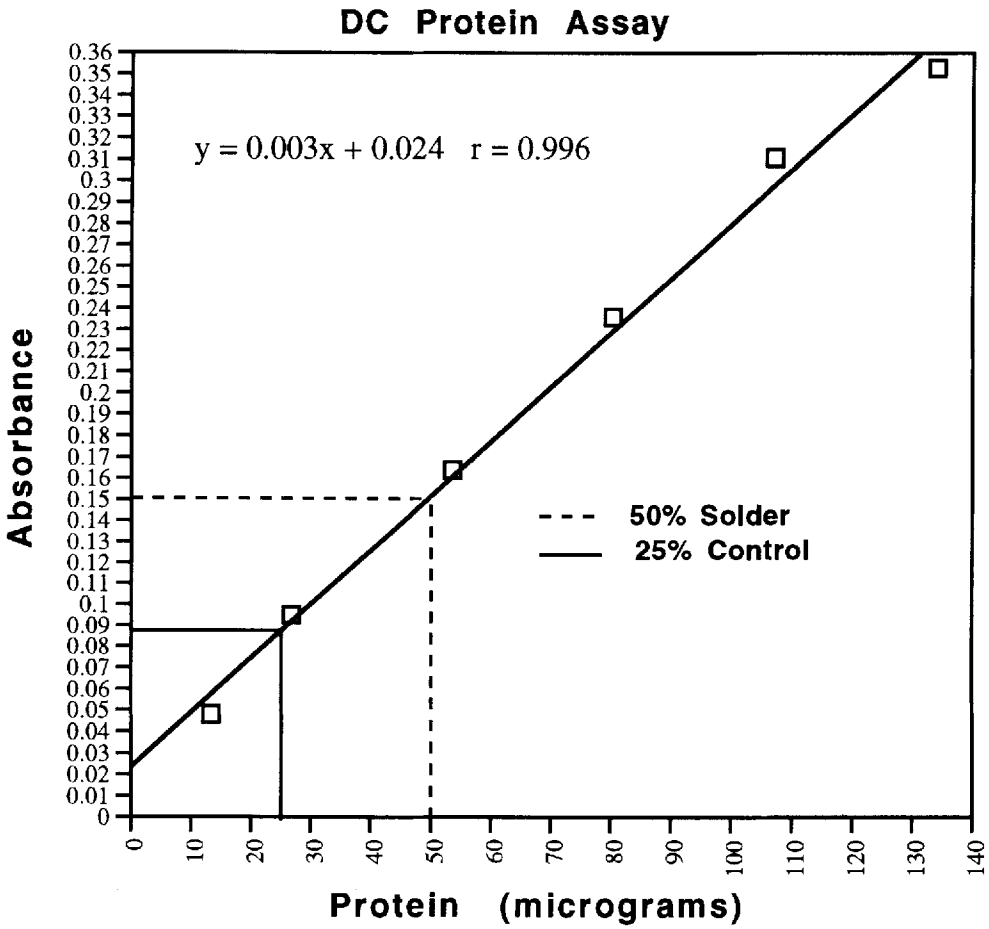

Fig. 3. Typical graph of protein concentrations comparing 25% control albumin with 50% albumin prepared using methods described in this report. Mean concentrations of the 25% and 50% were 25.12 (S.D. 0.25) and 50.1 (S.D. 1.37), respectively.

(Boekel Industries, Philadelphia, PA). Forty-four milliliters of each albumin sample were used to determine viscosity. All measurement were performed at a liquid temperature of 27°C (77°F). The number of seconds recorded from the time the liquid began to drain from the cup until a break occurred in the stream was recorded as "Zahn seconds." The following formula was used to convert Zahn seconds to centistokes (cSt):

V = K (T − C), where V = Kinematic Viscosity (cSt), T = Efflux Time (Zahn seconds), and K + C = Constants.

Protein Concentration Measurement

Protein concentrations were determined using the Bio-Rad DC Colorimetric Protein Assay (Bio-Rad, Hercules, CA). Aliquots of solder were analyzed using a Spectronic 601 spectrophotometer (Milton Roy, Rochester, NY) at 750 nm. Multiple assays were performed to determine consistency of solder preparation.

RESULTS

Polyacrylamide Gel Electrophoresis

The denaturing gel (Fig. 1) illustrates identical banding patterns of the 25% commercial human albumin (MW 66.5 kDa) compared to the albumin samples tested at various steps during the preparation of the 50% solution. The native albumin gel (Fig. 2) also reveals identical banding patterns of the test samples compared to the 25% control with an absence of bands below the 66 kDa band. The electrophoretic migration patterns confirm that the primary, secondary, and tertiary structural integrity of the human albumin remains intact during preparation.

Measurement of Viscosity

Viscosity of the 25, 40, and 50% albumin solutions was 18.7 cSt (±4) cSt), 88.65 cSt (±6), and 204.8 centistoke (± 4), respectively. These differ-

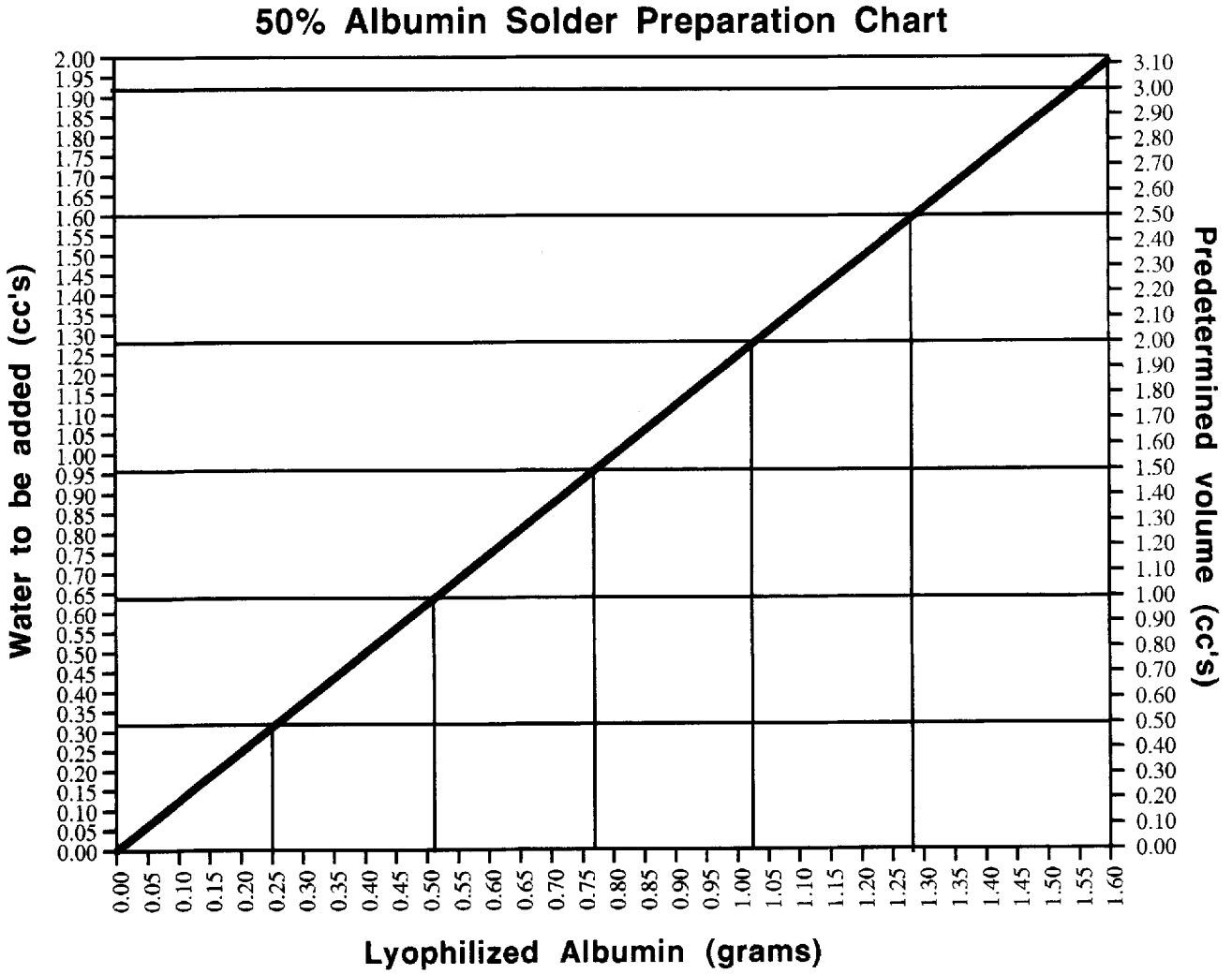

Fig. 4. 50% human albumin solder preparation chart. By selecting a predetermined final volume of solder on the yy axis, the amount of lyophilized albumin (x axis) and volume of water (y axis) needed to prepare the final solution are illustrated.

ences are statistically significant using paired t-tests with $P = .0001$.

pH Measurement

pH of the 50% albumin solution was determined to be 6.9 ± 0.5.

Colorimetric Assay for Protein Content

Protein concentration of the final 50% (w/v) solder solution was compared to a stock 25% solution. The mean protein concentration for the 25% and 50% solutions were 25.12 (S.D. = 0.25) and 50.12 (S.D. = 1.37), respectively. A typical graph illustrating protein concentrate plotted against absorbance is shown in Figure 3. The assay confirms that 50% albumin was prepared using the methods described above. The relationship between the weight of lyophilized albumin and the volume of distilled water required to reconstitute it to 50% is linear. As a result, we constructed a chart that permits the preparation of any predetermined final volume of 50% albumin to be made from a sterile stock of lyophilized albumin (Fig. 4).

Spectrophotometric Analysis

Spectrophotometric analysis was determined over the wavelength range between 400 to 820 nm. Peak absorption wavelengths for fluorescein, methylene blue, and ICG enhanced solders were determined to be 496 nm, 661 nm, and 805 nm, respectively (Fig. 5a–c). These peak wavelengths

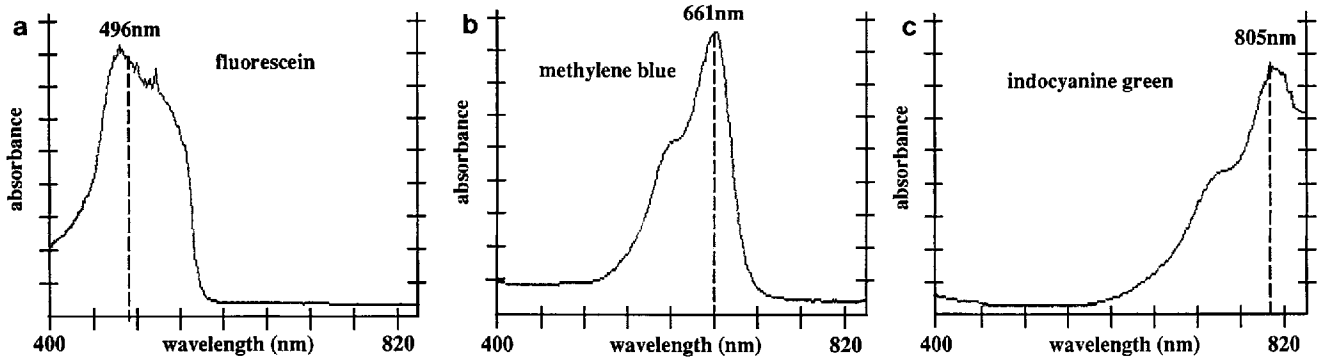

Fig. 5. (a–c) Peak absorption wavelengths (PAW) of chromophore enhanced solders. (a) fluorescein PAW = 496 nm, (b) methylene blue PAW = 661 nm, and (c) indocyanine green PAW = 805 nm. The peak absorption wavelengths of the chromophores remain unchanged when combined with high concentration albumin.

were unchanged from their predicted peak absorption wavelengths, confirming that the albumin does not shift this peak. Without the addition of chromophore, the solder did not show absorbance over this band of wavelengths.

DISCUSSION

The growing interest for using human albumin as a solder during laser tissue welding is based on several observations. It can be prepared as a single, large volume stock solution and stored in small aliquots to be used for many procedures. The solder can be stored without refrigeration for up to 3 years. Perhaps most importantly, the risk of bacterial contamination and transmission of the hepatitis virus or human immunodeficiency virus (HIV) has virtually been eliminated using techniques to prepare the commercially available, FDA-approved human albumin [16–19]. The commercial 25% human albumin used in the preparation of the 50% solder is a sterile, aqueous solution of albumin obtained from a large pool of adult human plasma by low temperature-controlled fractionation according to the Cohn process [20]. The albumin is heat stabilized during commercial preparation with N-acetyl tryptophan and sodium caprolate. Therefore it is possible to heat the albumin during processing to 60°C for 10 hours (pasteurization), thus achieving inactivation of viruses [18]. The safety of commercially prepared human albumin is so reliable that it rarely warrants discussion. As a result, infection from viruses is so infrequent as to constitute a reportable item [19].

Our lab has used 40% human albumin to perform laser tissue welding for the repair of urethral and ureteral tissues. We have found that repairs using human albumin solder are superior when compared with laser welding alone or to conventional suture closure [4,6,12,21]. Recently, our New York Hospital laboratory determined that the use of a 50% albumin solder resulted in a significantly higher acute bursting strength, when compared to lower concentrations [13]. As a result of this finding, we have developed a method to prepare a safe, sterile, viral free solder using commercially available human albumin.

Before laser tissue welding is performed in the clinical setting, we feel that it is our responsibility to optimize as many components of the process as possible. We have established that human albumin significantly improves postoperative results and that a 50% albumin solder performs best when compared to lower concentrations. Furthermore, a 50% concentration of solder is more viscous, preventing its migration prior to laser welding. This is especially important during laparoscopic applications where the tissue surface angles may not always be ideal.

We have completed studies using the 50% human albumin solder to repair large ventral defects in the canine urethra [22]. In this study, a thin (200 micron) layer of 50% human albumin was applied directly to the approximated tissue edges prior to welding with a 1.32 μm laser. Animals were followed for 96 days. A fifty percent fistula rate occurred in the suture control group (n=6) compared to a sixteen percent fistula rate in the laser solder group (n=6). We have begun clinical applications for the repair of hypospadias using a 50% human albumin solder. These surgical procedures are being performed under institutional IRB protocols (The New York Hospital).

We hope that the methods described in this report will help to improve overall confidence in the solders to be used in the clinical setting. However, it is important to emphasize that human albumin is *not* approved by the FDA for the specific indication as a laser tissue solder. Many steps including regulatory approval, clinical trials, and subsequently good manufacturing practices (GMP) will be required before any solder can be marketed for human use.

Future research should be directed toward making laser tissue reconstruction more efficient, safe, and less operator-dependent. One important question that remains to be answered is: How does the surgeon know that enough laser energy has been delivered to affect a satisfactory weld without causing excessive thermal tissue damage? Our group at Children's Hospital is currently testing a system for remote thermal controlled tissue welding [23]. This system provides instantaneous feedback of the tissue surface temperature to a computer that regulates the power output of the laser. When the predetermined tissue temperature at the weld site is reached, the laser power is automatically adjusted to maintain that temperature. This system will significantly reduce the training time for laser tissue welding and, for the first time, provide a means for quantitative, reproducible laser interaction. With continued research to optimize these techniques, laser welding promises to become a useful adjunct for reconstructive surgery, in general, and for laparoscopic tissue closure, in particular.

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